2410-Pos Board B102

Water Dynamics at Protein-Protein Interface: Molecular Dynamic Study of Virus-Host Receptor Complexes

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The dynamical properties of water in the condensed phase deviate from their bulk values at interfaces. Here we utilize all atom molecular dynamics (MD) simulations to characterize the dynamical properties of water present in protein-protein interfaces. We consider specifically the complexes formed between the attachment protein of the Nipah virus (G) and its preferred human receptors, ephrins B2 and B3. These protein-protein interactions constitute the first step in viral infection. X-ray diffraction of these two complexes indicates an exceptionally large amount of water crystallized in the interstitial region between the two proteins, as compared to other protein-protein complexes. Surprisingly despite a modest sequence identity of ~50% between the two ephrin receptors, the dynamical properties of water in the two complexes are qualitatively similar. Specifically, we find that the diffusion coefficients of interstitial waters are an order to magnitude smaller than those in bulk water. Nevertheless, while the water molecules in the interstitial region show a tendency to occupy their crystallographic positions, their residence times in the interstitial region are of the order of only a few hundred picoseconds. The water molecules in the interstitial region exchange with the bulk solvent, and only a tiny fraction ($< 5\%$) remain trapped in the interstitial region for more than 150 ns. In spite of the perpetual exchange with bulk media, the interstitial waters exhibit dipole relaxation rates and hydrogen bond lifetimes several orders in magnitude greater than bulk water. The waters in these two interfaces, in fact, carry a statistically large dipole moment perpendicular to the interface, which is perhaps in response to the multiple salt bridges present in the interface. Such deviations of water properties from bulk values are significantly different from those observed at lipid-water and protein-water interfaces.

2411-Pos Board B103

A Non-Active Site Set Domain Surface that is Crucial for Di-Methylation of Histone H3 Lysine 4 by the Mixed Lineage Leukemia-1 (MLL1) Core Complex

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Histone 3 (H3) lysine 4 (H3K4) methylation is an evolutionary conserved epigenetic mark that is involved in the control of gene expression. Lysine residues can be mono-, di-, or tri-methylated, often which each mark leading to different outcomes. In humans, the Mixed Lineage Leukemia-1 enzyme (MLL1, ALL1, HRX, KMT2A) is a SET1 family member that possesses H3K4 mono-methyltransferase activity conferred by its conserved Suppressor of Variegation, Enhancer of Zest, Trithorax (SET) domain. Di-methylation of H3K4 requires a sub-complex of proteins including WDR5, RbBP5, Ash2L, and DPY-30 (WRAD), which binds to MLL1 forming the MLL1 core complex. Di-methylation of H3K4 is associated with active transcriptional states of chromatin. We recently demonstrated that WRAD is a novel non-SET domain enzyme that catalyzes H3K4 di-methylation in a manner that is dependent on a non-active site surface from MLL1. The MLL1 surface required for interaction with WRAD and for H3K4 di-methylation is unknown. Genome sequencing studies recently revealed several missense mutations that occur in and around the SET domain in various cancers and developmental disorders. We noticed that a subset of missense mutations associated with human Kabuki syndrome (KS) map to a common non-active site surface in the MLL1 SET domain with no known function. In this investigation, we substituted all KS missense amino acid positions into MLL1 and observed that all are required for H3K4 di-methylation by the MLL1 core complex, which is associated with a loss of the ability of MLL1 to interact with the RbBP5-Ash2L heterodimer. Our results suggest that amino acids from this surface, called the Kabuki interaction surface or (KIS), are required for formation of the H3K4 dimethyltransferase active site within the MLL1 core complex.

2412-Pos Board B104

Identification of Protein-Protein-Interaction (PPI) Inhibitors and Stabilizers for Antimalarial Drug Development using SPR

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Malaria has been a human life threat for centuries, particularly in tropical and subtropical regions of the world. Nevertheless, our repertoire of medication to treat the disease has been very limited 1, and emerging resistance of the malaria parasite Plasmodium has further restricted the use of current antimalarial treatment. The most recent reports indicating artemisinin resistance in Cambodia2 are indeed alarming and underscore the critical importance of exploring novel pathways for interfering with the life cycle of the malaria parasite.

Surface plasmon resonance (SPR) has been a powerful tool to study protein ligand interactions. We utilize this technique for fragment and small molecule screening (~150-500 Da) to identify PPI-inhibitors or PPI-stabilizers 3.

In this study we present the identification of PPI-inhibitors for three plasmodial targets and two examples of PPI-stabilizers. We will furthermore demonstrate the in vitro effect on Plasmodium falciparum cultures of these PPI-inhibitors and PPI-stabilizers in the liver stage as well as blood stage form of the parasite. Small-molecules derived from our studies may represent useful probes for further dissecting the underlying biological mechanisms and analyzing the pathways in Plasmodium and other apicomplexan species such as Toxoplasma, Cryptosporidium, Eimeria, Theileria and Babesia. Further development of our current small molecules may provide initial hits for lead optimization in drug design studies.

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2413-Pos Board B105

New Microcalorimetric Methods for Measuring Ultratight Protein-Ligand Interactions

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Isothermal titration calorimetry (ITC) is the gold standard for the quantitative characterization of biomolecular interactions. However, reliable determination of the dissociation constant (K_D) is typically limited to the range 100 μM > K_D > 1 nM. Nevertheless, interactions characterized by a higher K_D can be assessed indirectly, provided that a suitable competitive ligand is available whose K_D falls within the directly accessible window. Unfortunately, the established competitive ITC displacement method has two major limitations. First, it requires the high-affinity ligand be soluble at high concentrations in aqueous solution (typically >100 μM). This poses serious problems in quantifying high-affinity interactions involving poorly water-soluble small-molecule ligands taken from compound libraries, as is usually the case in many drug-discovery projects. A second limitation of the displacement method is that the protocol necessitates at least two titrations to characterize one high-affinity inhibitor. This limits the great potential of microcalorimetry both in drug discovery efforts, where high sample throughput is required, as well as in basic research, where precious biological samples are often available only in limited amounts.

Here, we present two novel competition assays for measuring ultratight protein-ligand interactions by ITC that overcome these limitations. The first assay allows for a precise thermodynamic description of high-affinity protein-ligand interactions involving poorly water-soluble compounds (Krainer et al. *Anal. Chem.***2012**, *84*, 10715), while the second assay provides a simultaneous quantification of both the competitive, moderate-affinity and the high-affinity ligand in a single experiment with minimal sample requirement. We discuss the theoretical background of the approach and demonstrate some practical applications using examples of high-affinity protein-ligand interactions.

2414-Pos Board B106

Ru(II)Bis(2,2′ Bipyridine)L Complexes as Photorelease Agents for Bioactive Molecules: Photothermal Studies of Ligand Release

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The thermodynamics associated with small-protein interactions essentially define how drugs bind to target receptor proteins. As proteins are dynamic, it is of importance to understand the early time (sub-millisecond) thermodynamics and kinetics associated with small molecules binding. This time regime presents experimental challenges in both the measurement of the